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TITLE: "Assessing EphA2 and Ephrin-A as Novel Diagnostic and Prognostic Biomarkers of Prostate Cancer"

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"Assessing EphA2 and Ephrin-A as Novel Diagnostic and Prognostic Biomarkers of Prostate Cancer"

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This study seeks to evaluate EphA2 and ephrin-A1 as novel biomarkers of prostate cancer (PCa) diagnosis and/or prognosis. We are recruiting men at high risk for PCa who are undergoing prostate biopsy and prostatectomy at our institution. We will correlate their levels of EphA2 and ephrin-A1 mRNA as well as staining of phosphorylated pS897-EphA2 to the presence of PCa, the aggressiveness of PCa as determined by traditional clinical predictors, and race. Completion of the studies will achieve the following: 1) Novel biomarkers to improve the ability to distinguish between indolent and aggressive PCa; 2) More accurate prediction of disease outcomes to facilitate optimal treatment selection for each patient; 3) Elucidation of the biological mechanisms behind the PCa health disparities that affect minority men. During this last study period, we have enrolled 60 male patients into our study (60% African American, 35% Caucasian, 5% Asian). After receiving approval from the USAMRMC ORP HRPO, we have collected prostate tissue cores from each patient and are in the process of optimizing our protocol for RNA extraction and quantitative RT-PCR analysis (Specific Aims 1,2). Of these 60 patients, 14 (70% African American, 30% Caucasian) have undergone radical prostatectomy within the last study period, and we have obtained histologically confirmed benign and malignant prostate specimens from each. These specimens will be analyzed as previously described for Specific Aim 3.

15. SUBJECT TERMS
Prostate cancer; racial disparity

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1. **INTRODUCTION:**

   We seek to evaluate EphA2 and ephrin-A1, components of a receptor tyrosine kinase signalling pathway, as novel biomarkers of prostate cancer (PCa) diagnosis and/or prognosis. More accurate biomarkers are particularly important to African American men who are disproportionately affected by aggressive PCa and demonstrate a worse prognosis. We will recruit men at high risk for PCa who are undergoing prostate biopsy at MetroHealth Medical Center. Prostate tissue cores obtained from the biopsy procedure will be used for RNA extraction as well as protein staining. We will measure the levels of EphA2 and ephrin-A1 mRNA as well as staining of phosphorylated pS897-EphA2, which is a pro-oncogenic variant of the protein. These values will then be correlated to the presence or absence of PCa, the aggressiveness of PCa as determined by traditional clinical predictors, and race. Eventually, we will validate these data by performing the same analyses on full prostate specimens from enrolled patients who undergo radical prostatectomy. Completion of the proposed studies will achieve the following: 1) Novel biomarkers to improve our current ability to distinguish between indolent and aggressive PCa to determine which patients would benefit from therapy; 2) More accurate prediction of disease outcomes to facilitate optimal treatment selection for each patient; 3) Elucidation and potential countering of the biological mechanisms behind the PCa health disparities that affect African American men.

2. **KEYWORDS:** prostate cancer, biomarkers, racial disparity, health outcomes
3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**
   - **Specific Aim 1: Determine whether distinct levels and activity of EphA2 and ephrin-A distinguish benign from malignant prostate tissue and/or correlate with cancer aggressiveness.**
       - Major Task 1: Characterize EphA2 and ephrin-A expression levels in benign and malignant prostate tissue: 20% complete
       - Major Task 2: Characterize level of phosphorylated EphA2 in benign and malignant prostate tissue: 0% complete
       - Major Task 3: Correlate EphA2 and ephrin-A mRNA expression and/or protein staining with cancer aggressiveness: 0% complete

- **Specific Aim 2: Test the hypothesis that distinct expression and activity profiles of EphA2 and ephrin-A differentiate PCa in AA versus EA patients**
    - Major Task 1: Characterize EphA2 and ephrin-A expression levels between AA and EA men diagnosed with prostate cancer: 20% complete
    - Major Task 2: Characterize staining levels of phosphorylated EphA2 in AA versus EA men with prostate cancer: 0% complete
- **Specific Aim 3: Investigate whether EphA2 and ephrin-A are independent prognostic factors of PCa behavior and progression.**
    - Major Task 3: Correlate expression and activity levels of EphA2 and ephrin-A with clinical outcomes from a prospective cohort of PCa patients: 10% complete
- **What was accomplished under these goals?**
- Specific Aim 1, Major Tasks 1-3: For the first several months after the award start date of October 2014, I worked with my institution's IRB to bring the study in line with the requirements and recommendations of the Human Research Protection Office. My main objective during this period was to continue the recruitment of men undergoing prostate biopsy at MetroHealth Medical Center (MHMC) and collect prostate tissue cores for RNA extraction and analysis. To date, we have enrolled 60 men into the study. After receiving word of final HRPO approval for the protocol on July 29, 2015, we have begun RNA extraction from the prostate cores for the quantitative RT-PCR analyses. While waiting for HRPO approval, we tested methods of RNA extraction on prostate tissue specimens that had been collected prior to the beginning of this grant award. Trizol-based extraction of RNA seemed to produce the highest RNA yields and will be utilized to process our prostate biopsy core samples.
- Specific Aim 2, Major Tasks 1-2: These tasks are dependent on stratification of the data obtained in Specific Aim 1 by presence/absence of PCa and patient race. As such, we have not been able to progress within this Aim.
- Specific Aim 3, Major Tasks 1-3: We have begun enrolling patients into our proposed prospective cohort of men who undergo radical prostatectomy at MHMC. Out of the 60 men thus far enrolled into the study at the pre-diagnostic stage (i.e., before prostate biopsy), 14 men have been diagnosed with PCa and have undergone radical prostatectomy. From these 14 patients, we have obtained benign and malignant

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prostate tissue specimens. Once we have validated the efficiency of the RNA extraction and RT-PCR analyses from Aim 1, we will utilize the same methods on these tissue samples.

- **What opportunities for training and professional development has the project provided?**

- Since the start of the grant award, I have attended weekly lab meetings with Dr. Wang's group to discuss progress of my project as well as offer input into and derive insight from other related projects currently ongoing in the laboratory. Once a month, at minimum, I meet with Dr. Wang to discuss my progress and to plot future directions for the research as well as consider the type and timing of future grant applications. I also attend the Prostate Cancer Working Group Seminar Series and Journal Club, a monthly meeting of local prostate cancer researchers sponsored by the Cleveland Clinic. At this meeting, prostate cancer research scientists, both local and national, are invited to present their innovative work in the field, share their ideas, and discuss collaborative projects. I am also a member of the MetroHealth Cancer Center Oncology Research Committee, a group which meets monthly to discuss ongoing and prospective clinical trials at my institution. I am responsible for reviewing the trials related to urologic malignancies, include prostate, bladder, and kidney cancer. Lastly, I have engaged in extensive individual study and review of current literature on advances in prostate cancer diagnosis and treatment.

- **How were the results disseminated to communities of interest?**
- Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

- Our immediate plans are to optimize RNA extraction from prostate biopsy and prostatectomy tissue specimens and begin analysis of RNA and protein levels. Once we have enrolled at least 150 men into the study, we will correlate the data with the presence/absence of PCa, traditional clinical markers of disease aggressiveness (e.g., PSA level or Gleason grade), as well as race (African American versus European American).

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - Nothing to report.
   - **What was the impact on other disciplines?**
     - Nothing to report.
   - **What was the impact on technology transfer?**
     - Nothing to report.
   - **What was the impact on society beyond science and technology?**
     - Nothing to report.

5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
     - We have decided not to utilize an archival tissue bank of prostatectomy specimens from the Cleveland Clinic. We believe that, over the course of the grant award, we will be able to accrue sufficient patients into our own prospective prostatectomy cohort. We estimate we will have 70 to 100 men in the cohort by the end of the grant award.
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     - We encountered some delays with aligning our IRB consents and study forms with the requirements by the HRPO but final approval was obtained July 29, 2015.
   - **Changes that had a significant impact on expenditures**
     - Nothing to report
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
     - Nothing to report
   - **Significant changes in use or care of human subjects**
     - Nothing to report

6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**
     - Nothing to report
   - **Books or other non-periodical, one-time publications.**
     - Nothing to report
   - **Other publications, conference papers, and presentations.**

- ▪ Nothing to report
- ▪ **Website(s) or other Internet site(s)**
- ▪ Nothing to report
- ▪ **Technologies or techniques**
- ▪ Nothing to report
- ▪ **Inventions, patent applications, and/or licenses**
- ▪ Nothing to report
- ▪ **Other Products**
- ▪ Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- ▪ **What individuals have worked on the project?**

Name:	Carvell Nguyen
Project Role:	Principal investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	Dr. Nguyen has overseen the project and has been involved in the following facets of the project: patient recruitment, establishing and maintaining patient databases, molecular assays, data analysis, planning and performance of prostate biopsy, performance of radical prostatectomy, and patient follow-up.
Funding Support:	

Name:	Bing-Cheng Wang
Project Role:	Co-investigator/mentor
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	1.2
Contribution to Project:	Dr. Wang has provided scientific expertise, logistical support, and project feedback to the PI.
Funding Support:	NIH/NCI

Name:	Aaron Petty
Project Role:	Research assistant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Mr. Petty has been responsible for much of the benchwork requisite for the proposed studies, including RNA purification, RT-PCR analysis, and tissue IHC as well as processing and storage of the tissue specimens.
Funding Support:	

Name:	Elba Adriana Perez
Project Role:	Research coordinator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Ms. Perez has helped the PI with the clinical and logistical aspects of the project, including patient recruitment and consenting, transport of tissue samples between OR and the laboratory, and data collection/entry.
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
  - Nothing to report
- **What other organizations were involved as partners?**
  - Nothing to report

8. **SPECIAL REPORTING REQUIREMENTS**
  - Nothing to report

9. **APPENDICES:**
  - Nothing to report